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Note

Determination of bergapten and citropten in perfumes and suntan cosmetics by high-performance liquid chromatography and fluorescence

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Many furocoumarins found in several species of plant[1] are potent photosensitizing agents, known to have lethal and mutagenic effects[2]. Bergapten (5-MOP) is the major constituent of bergamot oil, widely used in the formulation of perfumes and suntan cosmetics. Like other psoralens, 5-MOP produces a phototoxic reaction in the skin after topical application and exposure to UVA radiation, followed by erythema and hyperpigmentation[3,4]. After exposure to the UVA radiation in sunlight 5-MOP stimulates melanin deposition, as occurs in the natural suntanning process.

Citropten or 5,7-dimethoxycoumarin (DMC) is found naturally in several citrus oils, including oils of bergamot, lime and lemon[5,7] in concentrations varying from 0.46 to 0.053%. DMC has been investigated as a potential substitute for 5-MOP in psoralen + UV-A therapy for treatment of psoriasis and mycosis fungoides[8–10].

Very recently Ashwood-Smith *et al.*[11] reported that the biological properties of DMC include dark-induced frame-shift mutagenesis in bacteria, lethal photosensitization and formation of sister chromatid exchanges in Chinese hamster cells.

As an alternative to other methods[12–15] we recently reported a high-performance liquid chromatographic (HPLC) procedure for the determination of 5-MOP in cosmetics based on the combined action of the column system and UV spectroscopy[16]. We have developed and refined the method to give more specificity by eliminating any interference of natural coumarins and furocoumarins and to obtain greater sensitivity with selective fluorescence detection of 5-MOP and DMC[18,19]. With this aim in mind, a procedure is presented here for a rapid and routine analysis of 5-MOP and DMC in various cosmetics, based on HPLC separation and subsequent fluorimetric detection by Latz and Ernes[17]. Each determination requires a total time of less than 10 min. Several randomly selected commercial products such as perfumes, eau de toilette, after-shave and suntan lotions with different degrees of protection were analyzed by a modified external standardization.

MATERIALS AND METHODS

5-MOP and DMC were supplied by the Institute of Pharmaceutical Chemistry, University of Padua.

A Perkin-Elmer series 3b liquid chromatograph equipped with a fully automated LS-4 fluorimetric detector and Sigma 15 data station was used. A Rheodyne

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7125 injector valve and ASV1 automatic switching valve were employed. Separation was achieved with an RP-8 (10 μm) Merck column. The analyses were performed at room temperature, using isocratic elution with a mobile phase of acetonitrile–water (35:65) at a flow-rate of 2.0 ml/min. The fluorimetric detector was set at excitation and emission wavelengths of 325/470 nm (5-MOP) and 335/435 nm (DMC), respectively. Standard solutions were prepared by diluting a stock solution of 5-MOP and DMC to concentrations ranging from 0.01 to 20 ng/μl in methanol. Perfumes and lotions were directly analyzed, whereas the emulsions (1.0 g) were treated using an appropriate solvent such as methanol, acetonitrile, methylene chloride or tetrahydrofuran (THF) and were passed through a 0.45-μm Millipore filter before injection.

RESULTS AND DISCUSSION

Stop-flow analysis was employed to obtain more accurate values for the excitation and emission wavelengths for 5-MOP and DMC, allowing for the effect of the mobile phase in the HPLC system (Fig. 1). All detections were carried out by setting the fluorimetric detector at wavelengths of excitation and emission depending on the native fluorescence of 5-MOP and DMC according to the relative retention time throughout the analysis. The effect of the injection volume on the resolution is important for peaks eluting close to the solvent front and for injections of a sample solvent stronger than the mobile phase. The reduced injection volume is preferable in routine analysis where large numbers of samples are handled.

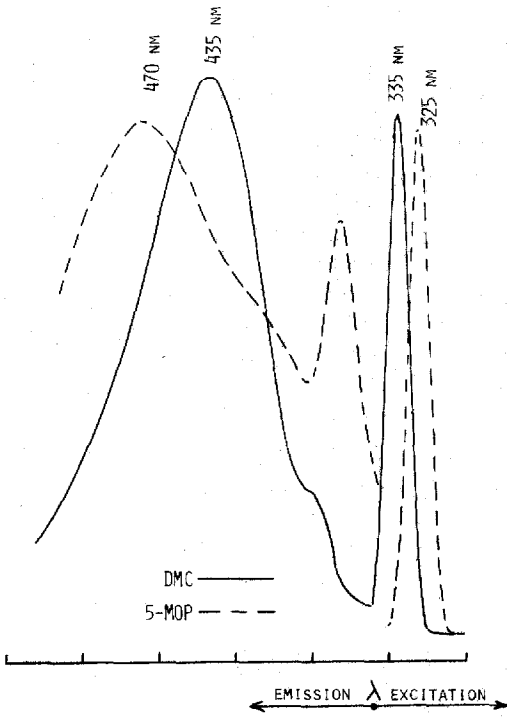

Fig. 1. Excitation and emission spectra of DMC and 5-MOP by effect of mobile phase obtained by stop-flow analysis.

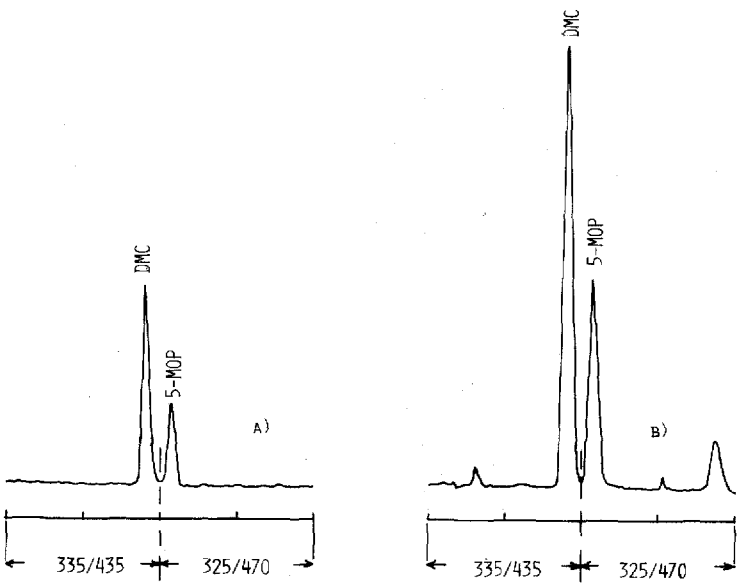

Fig. 2. Typical chromatograms of DMC and 5-MOP (A) in standard solution and (B) added to a suntan oil, obtained by LS/λ change.

Typical chromatograms of 5-MOP and DMC in standard solution and added to a cosmetic sample are shown in Fig. 2. Peaks were characterized by the standard addition method and by scanning the excitation and emission spectra of the peak eluted at the same retention time as the standard using the stop-flow technique.

The calibration curves of 5-MOP and DMC in standard solution and added

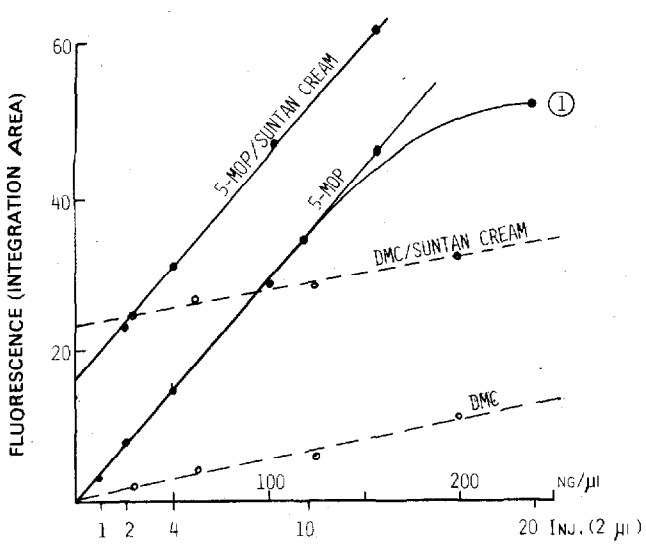

Fig. 3. Calibration curves of DMC and 5-MOP by standard solutions and by standard addition to a suntan cream sample (2 μl injection volume). 1 = Effect of injection volume on the linear calibration.

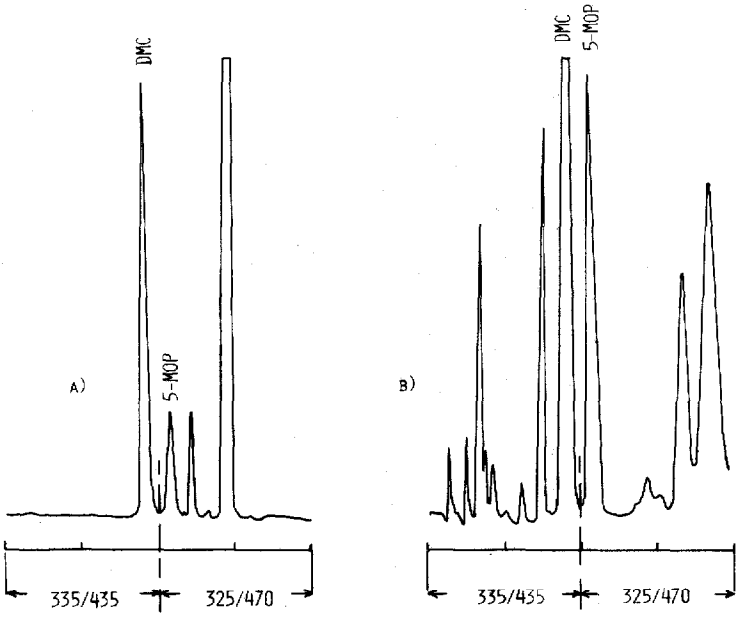

Fig. 4. Chromatograms of (A) a perfume and (B) a suntan cream in THF. Direct analysis with no sample pretreatment.

to a cosmetic sample are shown in Fig. 3. The similar slopes of these curves confirm the usefulness of the method proposed and the effect of the injection volume on the calibration linearity. Fig. 4 shows the chromatograms of two commercial cosmetic samples analyzed directly without pretreatment. For the emulsions, 5-MOP and DMC recovery and data reproducibility were excellent when THF was used as solvent in the pretreatment steps. This solvent allows almost complete solubilization and is particularly effective in lipophilic samples, for which a total preparation and analysis time of less than 10 min is required. Furthermore, the standard addition method appears an extremely valuable tool for selective fluorimetric measurements.

The results and chromatographic parameters summarized in Table I show that

TABLE I

HPLC PARAMETERS FOR 5-MOP AND DMC BY FLUORIMETRIC DETECTION

Data	*DMC*	*5-MOP*
Injection volume (μl)	2	2
Excitation/emission wavelength (nm)	335/435	325/470
Capacity factor (k)	6.4	7.7
Calibration standard	$y = 0.045x + 0.97$ $r = 0.99$	$y = 0.28x + 0.65$ $r = 0.98$
Calibration standard addition	$y = 0.041x + 23.8$ $r = 0.98$	$y = 0.27x + 16.1$ $r = 0.99$
Recovery (%)	97 ± 2 r.s.d.	98 ± 2 r.s.d.
Detection limit (pg)	1.0	10.0

the proposed method is a powerful technique for the rapid and sensitive detection of 5-MOP and DMC in cosmetics at the picomole levels.

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